Acta Medica Okayama

Volume 17, *Issue* 1 1963 *Article* 4

FEBRUARY 1963

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Abstract

A single subcutaneous injection of 20-methylcholanthrene into newborn AKR mice less than 24 hours old resulted in the acceleration of the development of lymphocytic leukemia, and induction of subcutaneous sarcomas and multiple-lung adenomas. Morphological descriptions of the respective tumors were given. It is suggested that the lungs of newborn mice of strain AKR may prove to be a sensitive organ to evaluate carcinogenicity of certain carcinogenic compounds.

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*PMID: 14078637 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 17, 51—61 (1963)

ACCELERATION OF THE DEVELOPMENT OF LEUKEMIA AND INDUCTION OF TUMORS IN AKR MICE BY NEONATAL INJECTION OF 20-METHYLCHOLANTHRENE

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Received for publication, February 19, 1963

Since the work of PIETRA *et al.*[1] who first reported induction of malignant lymphomas in Swiss mice by injecting 9, 10-dimethyl-1, 2-benzanthracene at birth, a number of papers have appeared in which newborn or infant mice were utilized as a sensitive biological system for chemical carcinogenesis.[2-12] Of these reports, recently, TOTH *et al.*[10] observed the accelerated development of malignant lymphomas in AKR mice injected at birth with 7, 12-dimethylbenz (a) anthracene. Adult Ak[13] and Aka[14] mice were also found to be susceptible to the accelerated development of leukemia by carcinogenic hydrocarbons. KAWAMOTO *et al.*,[15] on the other hand, reported that urethan slightly accelerated the onset of leukemia in adult mice of high-leukemic strains AKR and C58.

The present study was initiated with the purpose of investigating whether a virus is etiologically related to the leukemia or sarcoma induced in AKR mice by neonatal injections of 20-methylcholanthrene, and this paper describes multiple tumors thus induced in this strain of mice.

MATERIALS AND METHODS

AKR mice used in this experiment were inbred in our mouse colony from litters obtained in 1958 from the Baylor University College of Medicine, Houston, Texas. Newborn AKR mice less than 24 hours old were given a single injection of 0.05 ml. of 1% 20-methylcholanthrene solution in olive oil. The injections were performed with a tuberculin syringe and a fine (1/3 gauge) needle inserted subcutaneously in the interscapular region. The animals were housed in wooden boxes on wood shavings, and fed on Oriental laboratory chow and water *ad libitum*. They were sexed and weaned at four weeks of age.

For the transplantation of induced tumors, leukemic mesenteric tumor or subcutaneous sarcomatous tissue was snipped into small pieces and implanted by means of a trocar intraperitoneally or subcutaneously into adult mice of the same strain. The inoculated mice were observed for the growth of tumor at least during the next two months.

When the animals appeared moribund, they were sacrificed by cervical dislocation and examined post mortem. At the time of autopsy, the number of peripheral leukocytes were

counted, and imprint smears of the pertinent organs as well as blood smears were made to be stained with May-Grünwald-Giemsa stain. Representative sections were taken from each experimental animal. The tissues were processed routinely and stained with hematoxylin and eosin.

RESULTS

A total of three litters were subjected to neonatal injections of 20-methylcholanthrene. However, unfortunately, two litters were inadvertently lost during the early suckling periods and only one litter of seven mice formed the basis of this study. Of these seven mice, three males died of an intercurrent disease between 2 and 3 months of age and were too decomposed for histologic examination. Among the surviving four mice, there were 2 accelerated leukemias, 4 subcutaneous sarcomas, and multiple lung adenomas (Table I).

Table I. Tumors induced in newborn AKR mice given 0.5 mg. of 20-methylcholanthrene

Mouse #	Sex	Leukemia	Subcutaneous sarcomas	No. surface lung adenomas	Latency in days
1	F	Lymphocytic	Polymorphous-cell sarcoma	19	101
2	F	Lymphocytic		24	131
3	M		Fibrosarcoma	33	133
4	F		Spindle-cell sarcoma & rhabdomyosarcoma	60	143

Lymphocytic leukemia : As reported in our previous publication, 58 spontaneous leukemias in untreated AKR mice were observed between 6 and 15 months of age.[16] In the present experiment, therefore, leukemia developing before the age of 6 months was considered accelerated by the chemical carcinogen.

Accelerated lymphocytic leukemia was observed in two mice #1 and #2. In the mouse #1, grossly and microscopically, the leukemia was indistinguishable from spontaneous lymphocytic leukemia frequently seen in this strain of mice (Fig. 1). At the time of autopsy, the peripheral leukocyte count was 39,750 per cu. mm., with 5% lymphoblasts, 12% large lymphocytes, 55% small lymphocytes, 3% metamyelocytes, 21% segmented neutrophils, and 4% monocytes. There were 4 erythroblasts per 100 leukocytes. Postmortem examination revealed a large thymus, hepatosplenomegaly, and generalized lymphadenopathy. Microscopically, leukemic infiltration was seen in the liver (Fig. 2), spleen, thymus (Fig. 3), lymph node, lung, and kidney. Intraperitoneal transplantation of leukemic lymph node tissue of this mouse into two young adult AKR mice did not result in positive takes.

In the mouse #2, gross evidence of leukemia was questionable at autopsy, and both the differential and leukocyte counts were unremarkable.

On histologic examination, however, leukemic infiltration was found in the lung, kidney, and spleen. The leukemic cells were seen infiltrating some of the lung adenomas. The thymus was slightly enlarged and its imprint showed numerous lymphoblasts.

Subcutaneous sarcomas : A total of four subcutaneous tumors were produced; mice #1 and #3 developed one tumor and mouse #4 had two separate tumors. The subcutaneous tumors varied from 1.5 to 2 cm. in diameter and was located in the back at the general area where the carcinogen was deposited. They attached loosely to the skin but firmly to the underlying tissue. Necrosis of the tumor was observed in those of the mouse #4. The histologic picture differed considerably among these four tumors but showed a gerneral resemblance to carcinogen-induced subcutaneous tumors described by BONSER and ORR,[17] and HAAGENEN and KREHBIEL.[18]

The mouse #1 had a polymorphous-cell sarcoma which consisted of cells showing a great variation in size and shape. There were many giant cells. Some of these giant cells were definitely tumor cells but some were apparently derived from the degenerating muscle fibers (Fig. 5).

The subcutaneous tumor found in the mouse #3 was diagnosed as fibrosarcoma. The sarcoma was composed of slender, fusiform cells with vesicular nuclei. The cells were arranged in bundles running in many directions (Fig. 8). This tumor failed to grow when transplanted subcutaneously into two adult AKR mice.

The mouse #4 developed subcutaneously two independent tumors, which were diagnosed as spindle-cell sarcoma and rhabdomyosarcoma, respectively. The latter was characterized by the presence of many multinucleated giant cells with a number of hyperchromatic nuclei. Many of these giant cells were elongated and strap-shaped, and their cytoplasm stained deeply acidophilic and had a finely granular appearance (Fig. 9). No cross-striations could be identified. The rhabdomyosarcoma showed ulceration and secondary infection at the time of autopsy. Because of the morphological disparity, these two tumors were considered histogenetically unrelated. The spindle-cell sarcoma could not be successfully transplanted into adult AKR mice. All of the four sarcomas showed frequent mitotic figures.

Lung adenomas : The entire surface of the lungs were examined, after fixation in 10% formalin, for the presence and number of multiple nodules. Only the macroscopically visible nodules were counted. All the lobes of the lungs were studded with whitish nodules less than 2 mm. in diameter (Fig. 4). Occasionally, a few nodules coalesced to form a larger nodule. The nodules

located subpleurally were slightly elevated above the lung surface. Histologic examination revealed the usual type of adenomatous structure often described in the literature (Fig. 6).[19-22] The adenomas were composed of columnar or cuboidal cells with round or oval nuclei (Fig. 7). The growth was fairly solid but papillary processes were observed. Mitotic figures were practically absent, and there were no malignant changes noted. The uninvolved lung parenchyma was not remarkable. In some of the lung sections, different developmental stages of adenomas, from the focal accumulations of alveolar epithelium to the fully developed ones, were observed. This finding suggested the sequentional development of adenomas and coincided with the increasing number of lung nodules found in mice which survived longer (Table I).

For comparison, 11 AKR mice of both sexes between the age of 8 and 15 months were sacrificed and their lungs were examined grossly and microscopically. At the time of autopsy, 9 of them were found to have either a thymic lymphosarcoma or generalized leukemia. Sections were taken from two different lobes per animal. None of the 22 sections showed a single adenoma.

DISCUSSION

The incidence of spontaneous lymphocytic leukemia in normal AKR mice is very high and its peak incidence is attained at the age of 9 to 10 months.[16] When newborn AKR mice are injected with the leukemia virus isolated from spontaneous leukemias of the isologous mice, the time at death from leukemia can be considerably accelerated in the inoculated mice.[16,23-25] The present experiment and that of TOTH *et al.*[10] have demonstrated that chemical carcinogens can similarly hasten the development of leukemia in AKR mice. The chemical acceleration of leukemia, however, differs from the viral acceleration of leukemia in that, in addition to leukemia, other neoplasms such as subcutaneous sarcomas and lung adenomas can be induced simultaneously in many of the carcinogen-treated animals. In this experiment the mouse #1 showed three primary neoplasms—leukemia, subcutaneous sarcoma, and multiple lung adenomas—in one and the same body.

On the other hand, the effects of X-irradiation upon a high-leukemic strain of mice were investigated by REVERDY *et al.*,[26] and PORTEOUS,[27] and it was shown that the exogenous carcinogen was incapable of accelerating or augumenting the development of leukemia in newborn AKR or fetal AKR mice. This was rather unexpected in view of the proven increased susceptibility of very young animals to chemical carcinogens.

The mechanisms by which chemical carcinogens accelerate the onset of leukemia is totally unknown. Is the accelerated leukemia also viral in nature? If it is so, what about the etiology of subcutaneous sarcomas and lung adenomas?

Are they also transmissible by a filtrable agent? It is possible that only the accelerated leukemia is viral but not the other tumors. Another possibility is that all the tumors including the aceelerated leukemia are the manifestations of *de novo* chemical carcinogenesis, being etiologically unrelated to the leukemia virus indigenous to the AKR strain. Some of these problems are currently under investigation in our laboratory and the results will be reported elsewhere. Recently, SVOBODA[28] reported that electron microscopic study failed to visualize virus-like particles in any of the spontaneous and induced pulmonary adenomas in Swiss mice.

Because of the limited number of AKR mice used in the present experiment, it may not be possible to draw a definite conclusion. However, occurrence of multiple adenomas in the treated animals was striking. The pulmonary tumors were seen in all the four mice and the number of lung adenomas per animal ranged from 19 to 60 with an average of 34. This is indeed significant, since no lung adenomas were seen in any of the 11 control AKR mice older than 8 months. This negative finding along with the reports of FURTH and BARNES,[18] and TOTH *et al.*[10] that they observed no lung adenomas among control Ak or AKR mice provides sufficient evidence that strain AKR mice do not develop spontaneously tumors of the lung.

SHIMKIN[19] studied the susceptibility of seven strains of mice to pulmonary tumor induction by 20-methylcholanthrene and stated that the strain of mice which is most susceptible to the development of spontaneous pulmonary tumors is also most susceptible to the induction of these tumors with the carcinogen, and vice versa. In AKR mice, the situation is different; the strain is quite refractory to the development of spontaneous lung tumors and yet they appear to be highly sensitive to the lung tumor induction by the carcinogen.

SUMMARY

A single subcutaneous injection of 20-methylcholanthrene into newborn AKR mice less than 24 hours old resulted in the acceleration of the development of lymphocytic leukemia, and induction of subcutaneous sarcomas and multiple lung adenomas. Morphological descriptions of the respective tumors were given.

It is suggested that the lungs of newborn mice of strain AKR may prove to be a sensitive organ to evaluate carcinogenicity of certain carcinogenic compounds.

ACKNOWLEDGEMENT

The author wishes to express his thanks to Prof. K. Hiraki for his helpful advice, and to Emeritus Prof. Y. Hamazaki of the Department of Pathology for reviewing some of the histologic sections.

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EXPLANATION OF PLATES

Fig. 1. AKR mouse #1 wbich developed accelerated lymphocytic leukemia 101 days after a neonatal injection of 20-methylcholanthrene. This mouse also had a subcutaneous sarcoma and multiple lung adenomas.

Fig. 2. Liver of mouse #1 showing periportal and sinusoidal infiltration with leukemic cells. × 100.

Fig. 3. Thymic imprint of mouse #1 showing lymphoblasts. ×100.

Fig. 4. Multiple lung adenomas produced in mouse #4. Posterior view.

Fig. 5. Polymorphous-cell sarcoma produced in mouse #1. × 400.

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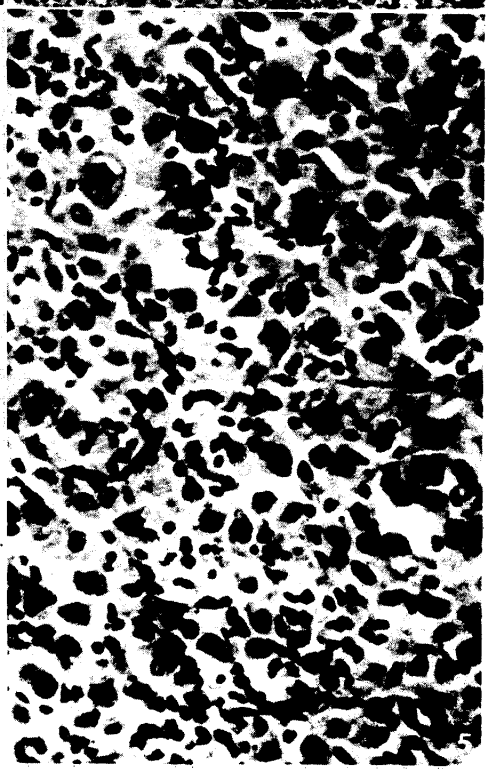

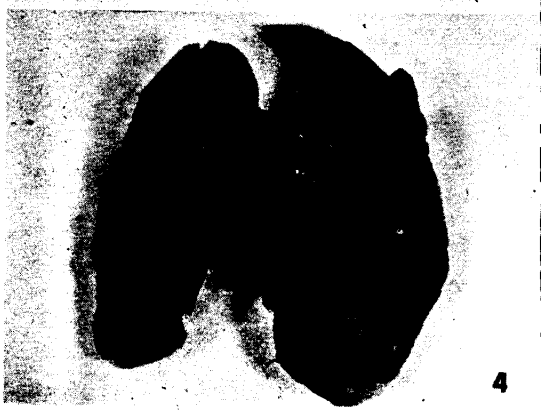

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Fig. 6. Histologic appearance of a lung adenoma in mouse # 4. ×100.
Fig. 7. Higher magnification of the lung adenoma shown in Fig. 6. × 400.
Fig. 8. Fibrosarcoma produced in mouse # 3. × 400.
Fig. 9. Rhabdomyosarcoma produced in mouse # 4. × 400.